Protocols for Test

E. coli Strains and Growth Media

E. coli Top10 was used for all the experiments and grown in Luria–Bertani (LB) medium or M9 minimal medium using glycerol as the carbon source. Kanamycin (10 μg/mL), ampicillin (50 μg/mL) and chloramphenicol (170 μg/mL) were added as appropriate.

Cultivation of *Microcystis aeruginosa*

Two strains of *Microcystis aeruginosa* were involved in our experiment： one was FACHB-1343, isolated from Lake Tai in 2010 by Yan Xiao, purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. While the other one is PCC7806, which was isolated, identified and kindly offered to us from the Institute of Hydrobiology, Chinese Academy of Sciences during our visit to Lake Tai Station in Wuxi. All samples were cultured in BG11 medium in conical flasks, and grew at 25 ℃ on a 12:12 h light/dark cycle controlled precisely by an illumination incubator.

The concentration test of *Microcystis aeruginosa*

The FACHB-1343 and PCC7086 samples were lysed by a noise isolating tamber, after being centrifuged for 10 min at 4000 rpm, supernatant was isolated and the absorbance of the supernatant was tested at 670nm thus represented for the concentration of *Microcystis aeruginosa*.

The killing efficiency of purified hen egg lysozyme

Parallel experiments were done to valid the killing efficiency of hen egg lysozyme. Hen egg lysozyme with the graded concentration of 2 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 500 μg/mL, 1000 μg/mL, and 2000 μg/mL were added in *Microcystis aeruginosa*. And the concentrations of *Microcystis aeruginosa* were tested.

Binding Assay

rTEV digestion assay

Culture the cells of Experimental group, C1 group, C2 group, C3 group in 5 mL LB medium. Stop the incubation when the OD600 reaches 0.8 and transfer 1 mL of cell culture of each group into a new 1.5 mL EP tube. Harvest the cells by centrifuging the cell cultures at 4000rpm for 4 min, discard the supernatant and leave the cell pellets at the bottom.

Prepare the rTEV buffer to the appropriate concentration. Add 1480 μL of rTEV buffer and 20 μL of rTEV protease into the EP tube with cell pellet inside. Mix the cells and the reagents afterwards and start rTEV digestion at 30℃ for 2 hours.

Centrifuge the EP tube at 2000 rpm for 6 min, and centrifuge again at 4000 rpm for 4 min

directly afterwards to form the cell pellet at the bottom. Transfer the supernatant to a new EP tube and measure the fluorescence intensity using microplate reader (excitation wavelength: 584 nm; absorbance wavelength: 610 nm)

MVN-*M. aeruginosa* binding assay

Thaw the purified MVN protein on ice, and add protein buffer during the procedure to prevent partial precipitation of the protein. Dilute the protein solution with protein buffer to the concentration of 0.1 mg/mL

Transfer 1 mL of cyanobacteria culture to an EP tube (OD670 of the lysed cyanobacteria is controlled to 0.45-0.50). Wash the cyanobacteria with PBS and resuspend it to 1 mL for 1 time.

Use mild sonication to break the colonies formed for 5 times. Then apply 20 μL of cyanobacteria culture onto the glass slide and fix by drying at RT. Cover the cells with 20 μL of protein solution and incubate at RT for 30 min.

Move the solution with filter paper carefully. Later, wash the glass slide with 100 μL of PBS for 3 times and carefully draw off the remaining PBS with filter paper. Finally, view the cells under fluorescence microscope.

***E. coli* - *M. aeruginosa* binding assay**

Incubate the *E. coli* cells overnight for 12 h in order to activate the cells. Transfer *E. coli* culture into a new LB medium (Chloramphenicol added) and incubate it till the OD600 reaches 0.6. Then, transfer 1 mL of cyanobacteria culture to an EP tube (OD670 of the lysed cyanobacteria is controlled to 0.45-0.50). Also transfer 1 mL of *E. coli* culture to another EP tube. Wash both of the cyanobacteria and E. coli with PBS and resuspend them to 1 mL again for 1 time.

Use mild sonication to break the cyanobacteria and *E. coli* colonies formed for 5 times and mix 45 μL of cyanobacteria culture, 50 μL of *E. coli* culture and 5 μL of PBS in a new EP tube. Incubate the co-culture for 1 h at 30 ℃.

After incubation, apply 20 μL of the co-culture onto a glass slide and wait for it to dry up at RT. Add 4 μL of PBS onto the dried co-culture and observe under fluorescence microscope lastly.

Microcystin Assay

Caliberation curve of PP1

0.2 to 1 unit of protein phosphate 1(PP1, New England Biolab) was diluted in 20ul NEB PMP buffer and mixed with 180uL of 15mM pNPP dissolved in NEB PMP buffer. The microwell plate(costar 3603 96 well assay plate, Corning Incorporated) containing the reaction system was incubated at 30 ℃. The result was monitored by measuring the absorbance at 405nm every minute for 12 hours with a thermo plate reader.

MlrA Inhibition assay

0.5 unit protein phosphate 1(PP1, New England Biolab) was diluted in 10ul NEB PMP buffer. 10ul of sample containing Microcystin-LR(MC-LR)of the concentration from 1μg/L to

100μg/L was also diluted in the same buffer. PP1 and sample were mixed and incubated at room temperature for 5min and 180uL of 15mM pNPP dissolved in NEB PMP buffer was added. The microwell plate(costar 3603 96 well assay plate, Corning Incorporated) containing the reaction system was incubated at 30 ℃. The result was monitored by measuring the absorbance at 405nm every minute for 12 hours with a thermo plate reader.

MlrA activity assay

A degradation assay was performed for heterologous strains. E. coli. which carried pET21(+)a-mlrA plasmid, pET21(+)a-pelB-mlrA plasmid and pET21(+)a-TorA-mlrA plasmid were incubated with MC-LR at a final concentration of 100ng/L at 25 ℃. Cells carrying empty vector were used as a negative control. Same bacteria solution were centrifugalized and treated by same volume of lysozyme solution(0.5mg/mL lysozyme, 30mmol/L Tris-Cl, 20%(m/V) sucrose). Same concentration of MC-LR was added to the solution. After 12 hours, 36 hours and 72 hours, all the reaction was terminated by heating to 98 ℃. Samples were cooled, diluted 4 times and analysed by PP1 inhibition assay method.